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AUTHOR(S):

Hanai, Tetsuya; Asami, Koji; Koizumi, Naokazu

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Dielectric Theory of Concentrated Suspensions of Shell-Spheres in Particular Reference to the Analysis of Biological Cell Suspensions

Tetsuya HANAI, Koji ASAMI, and Naokazu KOIZUMI*

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A theoretical formula for interfacial polarization is presented with which to express the dielectric behavior of concentrated suspensions of spherical particles covered with a shell phase. The formula is of a compound form of Wagner's and Bruggeman's equations. A computing scheme is given to carry out numerical calculations of the formula as a function of complex variables. By the use of the computing scheme, numerical calculations of the formula were performed with a set of phase parameters which are in conformity with biological suspensions of conducting spheres covered with a very thin and nonconducting membrane. The resulting complex plane plots of the complex permittivity showed characteristic profiles different from circular arcs as well as from semicircles, especially at higher concentrations. The procedure of fitting the theoretical curve to the observed relative permittivities is proposed so that the membrane capacitance and the relative permittivity and the conductivity of the inner phase may be evaluated. An example of the curve-fitting is shown with the dielectric data observed for an erythrocyte suspension.

KEY WORDS: Interfacial polarization / Membrane capacitance / Membrane permittivity / Inner phase conductivity / Plasma phase conductivity / Erythrocyte suspension /

I. INTRODUCTION

It is known that biological suspensions of erythrocytes, bacterial cells, and yeasts show a dielectric relaxation due to the interfacial polarization.¹⁻⁹⁾ From a dielectric point of view, such suspensions are in a triphasic structure: the conducting cytoplasm covered with the poorly conducting shell phase of lipidic membranes are dispersed in a conducting continuous medium. An elaborate dielectric theory of interfacial polarization for such triphasic systems in concentric structure was proposed by Pauly and Schwan,¹⁰⁾ and made it possible to evaluate membrane capacitance, relative permittivity (dielectric constant) and conductivity of the inner plasma-phase from the observed data on dielectric relaxations of biological cell suspensions. The general equation of this theory was derived from an analysis of quasi-electrostatic field, and has a functional form which is also expressed with repeated application of Wagner's equation proposed for diphasic suspensions: the first is for a sphere composed of the spherical inner phase and the concentric shell phase, and the second for the sphere and the outer medium.

For diphasic systems such as oil-in-water and water-in-oil emulsions, it was

* 花井哲也, 浅見耕司, 小泉直一: Laboratory of Dielectrics, Institute for Chemical Research, Kyoto University, Uji, Kyoto.

reported by many workers¹¹⁻¹³) that Wagner's equation is in poor agreement with experiments especially at higher concentrations of the suspending particles, and that Bruggeman's equation is satisfactory for representing observed data. The expressions in Wagner's type, therefore, might be insufficient quantitatively also for the theoretical treatment of the triphasic system proposed by Pauly and Schwan especially at higher concentrations of the suspending particles.

In this paper a dielectric theory of interfacial polarization is proposed to express the dielectric behavior of concentrated suspensions of spherical particles covered with a shell phase. Since the theoretical expression derived is of a complicated form including complex variables, a computing scheme is presented to obtain the numerical solutions. Some features of the theory are pointed out regarding the type of dielectric relaxation. Finally the procedure of curve-fitting is described with which to evaluate the membrane capacitance of the shell phase, the relative permittivity and the conductivity of the inner phase from observed data.

II. SYMBOLS

ϵ	relative permittivity or dielectric constant
κ	electrical conductivity
ϵ_0	permittivity of free space
ϵ^*	complex relative permittivity or complex dielectric constant
$\bar{\epsilon}_s^*$	equivalent complex relative permittivity for a shell-sphere
f	measuring frequency
f_0	characteristic frequency at which the loss factor shows the maximum
D	diameter of inner phase
d	thickness of shell phase
ϕ	volume fraction of spheres with a shell in suspension
C_M	specific membrane capacitance for shell phase

Subscripts

a	outer phase
s	shell phase
i	inner phase
l	limiting value at low frequencies
h	limiting value at high frequencies

III. THEORY OF INTERFACIAL POLARIZATION FOR CONCENTRATED SUSPENSION OF SPHERES COVERED WITH A SHELL PHASE

A problem considered here is to derive a complex relative permittivity ϵ^* of a concentrated suspension that spheres (the complex relative permittivity ϵ_i^* , the diameter D) covered with a shell phase (ϵ_s^* , the thickness d) are dispersed in a continuous medium (ϵ_a^*) with a high volume fraction ϕ , as shown in Fig. 1-A. According to a consideration carried out first by Maxwell¹⁴ for quasi-electrostatic field, the equivalent complex relative permittivity $\bar{\epsilon}_s^*$ (Fig. 1-C) of a diphasic system in concentric structure shown in Fig. 1-B is given by

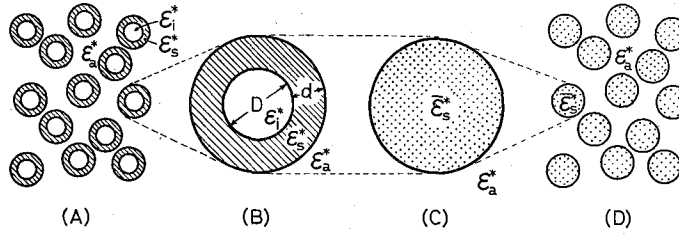


Fig. 1. Schematic diagram for a theoretical model of a concentrated suspension of shell-spheres. (A) Concentrated suspension of spheres (ϵ_i^*) covered with a shell phase (ϵ_s^*) dispersed in a continuous medium (ϵ_a^*). (B) Shell structure of a suspending particle. (C) A homogeneous sphere ($\bar{\epsilon}_s^*$) as an equivalent of the shell sphere shown in (B). (D) Concentrated suspension of particles ($\bar{\epsilon}_s^*$) dispersed in a continuous medium (ϵ_a^*) as an equivalent of the suspension shown in (A).

$$\bar{\epsilon} = \epsilon_s^* \frac{2\epsilon_s^* + \epsilon_i^* - 2(\epsilon_s^* - \epsilon_i^*)[D/(D+2d)]^3}{2\epsilon_s^* + \epsilon_i^* + (\epsilon_s^* - \epsilon_i^*)[D/(D+2d)]^3} \quad (1)$$

irrespective of ϵ_a of the outer phase.

Next an expression must be derived for the relative permittivity (ϵ^*) of a disperse system shown in Fig. 1-D, where the spheres ($\bar{\epsilon}_s^*$) are suspended in a continuous medium (ϵ_a^*) with a high volume fraction Φ . For diphasic systems such as emulsions, it was found that Wagner's equation¹⁵⁾ does not hold in higher concentrations. Hence Hanai^{16,17)} derived an equation in Bruggeman's type extended to complex numbers by means of successive applications of Wagner's equation to the infinitesimally increasing processes in concentration of the disperse phase. In a similar manner, it can readily be shown that complex relative permittivity ϵ^* of the present suspension illustrated in Fig. 1-D is given by

$$\frac{1}{1-\Phi} \frac{\epsilon^* - \bar{\epsilon}_s^*}{\epsilon_a^* - \bar{\epsilon}_s^*} \left(\frac{\epsilon_a^*}{\epsilon^*} \right)^{1/3} = 1. \quad (2)$$

Thus the complex relative permittivity ϵ^* of the concentrated suspensions can be expressed as a function of ϵ_a^* , ϵ_s^* , ϵ_i^* , D , d , and Φ , where

$$\epsilon^* = \epsilon - j \frac{\kappa}{2\pi f \epsilon_v}, \quad (3)$$

$$\epsilon_a^* = \epsilon_a - j \frac{\kappa_a}{2\pi f \epsilon_v}, \quad (4)$$

$$\epsilon_s^* = \epsilon_s - j \frac{\kappa_s}{2\pi f \epsilon_v}, \quad (5)$$

$$\epsilon_i^* = \epsilon_i - j \frac{\kappa_i}{2\pi f \epsilon_v} \quad (6)$$

and

$$j = \sqrt{-1}.$$

IV. COMPUTER EXPERIMENTS ON NUMERICAL SOLUTIONS OF THE EQUATION

In the present study numerical calculations were performed with a Hewlett-Packard Model 9810A Programmable Calculator. Since Eq. (2) has the compli-

cated form including a cubic root of the complex variable, the numerical solutions were obtained by the following computing scheme which is within the reach of the present calculator.

- (a) By the use of subroutine programmes of addition, subtraction, multiplication, and division for complex numbers, $\bar{\epsilon}_s^*$ of Eq. (1) can readily be calculated numerically provided that the phase parameters ϵ_s^* , ϵ_i^* , D , and d are given.
- (b) The values of $\bar{\epsilon}_s^*$ thus obtained are substituted for Eq. (2) to carry out the subsequent calculation for ϵ^* .
- (c) By cubing the both sides of Eq. (2), we have a cubic equation with respect to ϵ^* as

$$\epsilon^{*3} - 3\bar{\epsilon}_s^* \epsilon^{*2} + \left\{ 3\bar{\epsilon}_s^{*2} + \frac{[(\emptyset - 1)(\epsilon_a^* - \bar{\epsilon}_s^*)]^3}{\epsilon_a^*} \right\} \epsilon^* - \epsilon_a^{*3} = 0. \quad (7)$$

The coefficients of the cubic Eq. (7) can be determined by substituting ϵ_a^* , $\bar{\epsilon}_s^*$, and \emptyset .

- (d) By the use of a computer subroutine programme "Root-finder of cubic equation with complex numbers", we obtain three roots of Eq. (7) designated by ϵ_1^* , ϵ_2^* , and ϵ_3^* .
- (e) Among the three roots ϵ_1^* , ϵ_2^* , and ϵ_3^* , we have to choose only one solution satisfying Eq. (2) by means of the following criterion. Since Eq. (7) is derived by cubing Eq. (2), respective substitutions of the three roots ϵ_1^* , ϵ_2^* , and ϵ_3^* into a function

$$F(\epsilon^*) = \frac{1}{1 - \emptyset} \frac{\epsilon^* - \bar{\epsilon}_s^*}{\epsilon_a^* - \bar{\epsilon}_s^*} \left(\frac{\epsilon_a^*}{\epsilon^*} \right)^{1/3} \quad (8)$$

are to give

$$F(\epsilon_1^*) = 1 + 0 \cdot j = \exp(0 \cdot j), \quad (9)$$

$$F(\epsilon_2^*) = -\frac{1}{2} + \frac{\sqrt{3}}{2}j = \exp\left(\frac{2\pi}{3}j\right) \quad (10)$$

and

$$F(\epsilon_3^*) = -\frac{1}{2} - \frac{\sqrt{3}}{2}j = \exp\left(-\frac{2\pi}{3}j\right) \quad (11)$$

respectively. Here the exponent $1/3$ in Eq. (8) denotes a principal value of cubic roots. When each of the three roots is thus substituted into $F(\epsilon^*)$ given by Eq. (8), the roots which lead to either Eq. (10) or Eq. (11) should be ruled out, and only one root giving Eq. (9) is adopted as the solution of Eq. (2).

- (f) The only root is regarded as ϵ^* of Eq. (2), giving the values of ϵ , κ , and ϵ'' for the concentrated disperse system.

V. THE COMPLEX PERMITTIVITY PROFILE OF THE THEORY

A general equation of Pauly and Schwan's theory¹⁰⁾ was characterized by two relaxation times. Under the conditions $d \ll D$, $\kappa_s \ll \kappa_a$ and $\kappa_s \ll \kappa_i$, which are usually the case for biological cell suspensions, their equation reduced in effect to a single relaxation system.¹⁸⁾ In this instance, the complex plane plots of the complex relative permittivity were simplified to semicircles.

On the contrary, Eq. (2) of the present theory cannot be simplified even under the similar conditions. To find a clue to the permittivity profile, numerical calculations were carried out following the procedure shown in the preceding section by

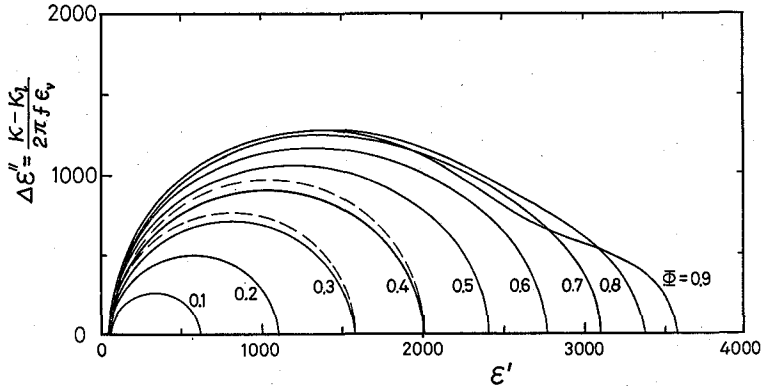


Fig. 2. Complex plane plots of complex relative permittivity for suspensions of shell-spheres calculated from Eq. (2) (solid curves). The dashed curves are semicircles. Phase parameters used: $\epsilon_a=80$, $\kappa_a=\kappa_i=2.5$ mS cm⁻¹, $\epsilon_i=50$, $\epsilon_s=6.5$, $\kappa_s=0$ mS cm⁻¹, $d=50$ Å, $D=3.8$ μm.

using a set of phase parameters relevant to biological cell suspensions. Some examples of the results are depicted in Fig. 2.

The profiles show marked deviations from circular arcs as well as from semicircles. At low concentrations ($\Phi < 0.3$) the profiles seem to be close to semicircles, the characteristics being the same as the results of Pauly and Schwan's theory. At medium concentrations ($\Phi = 0.3 \sim 0.5$), the profiles may be approximated by circular arcs proposed by Cole and Cole,¹⁹⁾ the examples being found in various biological suspensions.^{2, 20-25)} At high concentrations ($\Phi = 0.6 \sim 0.8$), the profiles are seen to show remarkable deviations from circular arcs. At extremely high concentrations ($\Phi = 0.8 \sim 0.9$), the profiles show very peculiar patterns of which the lower frequency part might be simulated by another relaxation process. As a matter of fact, such high concentrations ($\Phi = 0.8 \sim 0.9$) exceed a state for the close-packed structure with uniform spheres, the examples being unavailable in biological suspensions.

VI. PROCEDURE TO DETERMINE THE PHASE PARAMETERS BY CURVE-FITTING BASED ON THE EQUATION

Owing to the complicated functional form of Eq. (2), it is impossible to calculate straightforwardly the phase parameters such as Φ , ϵ_s , ϵ_i , and κ_i by using dielectric parameters ϵ_l , ϵ_h , κ_l , and f_0 observed. The determination of the phase parameters, however, is possible by fitting the theoretical curve of Eq. (2) to the observed data. Prior to the curve-fitting, it is advisable to make a list of the effects of changing one of these phase parameters on the change in the dielectric parameters predictable from Eq. (2). The procedure is the same as that discussed¹⁸⁾ previously for Pauly and Schwan's theory. The numerical considerations are hereafter restricted to the case of $\kappa_s < 10^{-4} \times \kappa_a$ or preferably $\kappa_s = 0$, which is pertinent to biological suspensions.

The calculation was made, on a reference state specified by a set of phase parameters relevant to biological suspensions, by changing one of the phase parameters in Eq. (2). The relative variations in the dielectric parameters from those for the

Table I. Response of Characteristic Dielectric Parameters to Individual Changes in Phase Parameters

Case	phase parameter [% change]	Variation from values for the reference state ^{a)} , % Dielectric parameter			
		κ_l	ε_l	ε_h	f_0
I	Φ [+10%]	-6.4	+ 3.9	- 1.3	- 1.2
II	ε_s [-50%]	0.00	-33	- 7.1	+58
III	ε_i [-50%]	0.00	0.00	-11	+12
IV	κ_i [-50%]	0.00	0.00	0.00	-41

a) The reference state is specified: $\varepsilon_a = \varepsilon_i = 80$, $\kappa_a = \kappa_i = 15 \text{ mS cm}^{-1}$, $\varepsilon_s = 3$, $\kappa_s = 0 \sim 10^{-4} \times \kappa_a$, $d = 50 \text{ \AA}$, $D = 0.5 \text{ }\mu\text{m}$, and $\Phi = 0.3$.

$$\text{Variation} = \frac{\left(\begin{array}{c} \text{Value by Eq. (2) after changing} \\ \text{one of phase parameters} \end{array} \right) - \text{Value for the reference state}}{\text{Value for the reference state}} \times 100$$

reference state are listed in Table I. The general feature found in the Table is very similar to the case of Pauly and Schwan's theory in that all six terms in the lower-left part of the Table are zero. Thus the procedure to determine the phase parameters can be stated as follows:

Step 1 To put tentatively $\varepsilon_i = \varepsilon_a$, $\kappa_i = \kappa_a$, $\varepsilon_s = 3$, and $\kappa_s = 0$, provided that ε_a and κ_a are given from direct measurements of the continuous medium.

Step 2 To find a proper value of Φ so that the calculated value of κ_l may fit in with the observed value of κ_l .

Step 3 To find ε_s so that the calculated ε_l may fit in with the observed ε_l .

Step 4 To find ε_i so that the calculated ε_h may fit in with the observed ε_h .

Step 5 To find κ_i so that the calculated f_0 may fit in with the observed f_0 .

If the fitting to ε_l and ε_h turned out insufficient after *Step 5* was carried out, *Steps 3, 4, and 5* should be repeated. The numerical values presented in Table I is effective for this reference state. Strictly speaking, such a survey table must be prepared on a reference state pertinent to the respective cases, though the general features in Table I necessary for the curve-fitting procedure is in fact subjected to no alteration.

VII. EXAMPLE OF THE APPLICATION

An example of the application of the procedure proposed is shown with a biological suspension. A bovine erythrocyte suspension was prepared by washing the blood thrice with a saline solution after addition of heparin, and finally being suspended in a 50 mM NaCl solution containing 250 mM sucrose for adjusting the tonicity. The measured relative permittivities are shown in Fig. 3.

The respective parameters subjected to *Steps 1 to 5* are summarized stepwise in Table II. The associated frequency profiles of the relative permittivity are illustrated in Fig. 3. From this curve-fitting the following phase parameters were evaluated: $\Phi = 0.540$, $\varepsilon_s = 4.39$, $\varepsilon_i = 69.0$ and $\kappa_i = 2.84 \text{ mS cm}^{-1}$. Hence the membrane capacitance was calculated to be $C_M = 0.777 \text{ }\mu\text{F cm}^{-2}$, from a relation

$$C_M = \frac{\varepsilon_s \varepsilon_i}{d} \frac{1 + 2d/D}{(1 + d/D)^2} \quad (11)$$

The erythrocytes measured are, to speak strictly, in a form of depressed sphere

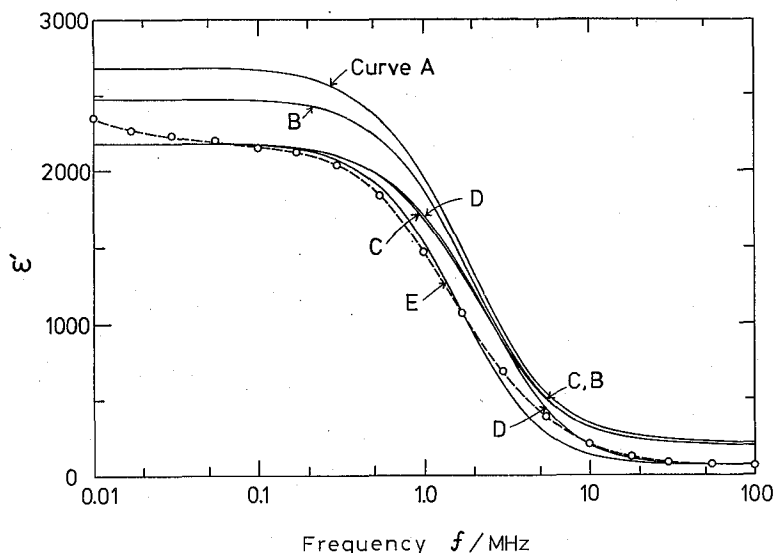


Fig. 3. Frequency dependence of relative permittivity and conductivity for a bovine erythrocyte suspension and the procedure of curve-fitting. The hollow circles (—○—) are the measured values. The curves A, B, C, D, and E correspond to Steps 1, 2, 3, 4, and 5 in Table II, respectively. The observed values for the outer medium are: $\epsilon_a=77.1$, $\kappa_a=4.346 \text{ mS cm}^{-1}$.

Table II. Variations of Dielectric Parameters Associated with the Variation of the Phase Parameters Subjected to Each Fitting Step

Curve-fitting procedure	Curve in Fig. 3.	Phase parameter				Dielectric parameter			
		ϕ	ϵ_s	ϵ_i	κ_i mS cm^{-1}	κ_l mS cm^{-1}	ϵ_l	ϵ_h	$f_{1/2}^{a)}$ MHz
							Observed value		
						1.356	2176	71.5	1.63
Step 1 ^{b)}	A	0.6	5.00	400	4.346	1.099	2681	197	1.79
Step 2	B	0.540	5.00	400	4.346	1.356	2475	180	1.83
Step 3	C	0.540	4.39	400	4.346	1.356	2176	178	2.07
Step 4	D	0.540	4.39	69.0	4.346	1.356	2176	71.5	2.23
Step 5	E	0.540	4.39	69.0	2.840	1.356	2176	71.5	1.63

a) Since f_0 is difficult to be assessed in observed data, the fitting was carried out with $f_{1/2}$ which is the frequency for a half-value point of the entire dielectric dispersion. At lower concentrations ($\phi < 0.6$), Eq. (2) shows no discernible difference between f_0 and $f_{1/2}$.

b) Values of the phase parameters at the starting state (Step 1) are: $\epsilon_a=77.1$, $\kappa_a=\kappa_i=4.346 \text{ mS cm}^{-1}$, $\epsilon_s=5$, $\kappa_s=0 \text{ mS cm}^{-1}$, $d=50 \text{ \AA}$, $D=4.75 \text{ }\mu\text{m}$, $\phi=0.6$, and $\epsilon_i=400$.

Owing to exaggerating the difference between Curves A and D in Fig. 3, an extraordinarily large value $\epsilon_i=400$ was adopted.

different from perfect sphere which is assumed in the present theory. Such a kind of non-spherical features must be discussed further. Nevertheless, in the present paper, the experimental data of erythrocyte suspensions were cited as a mere example to show the practice of the fitting procedure proposed.

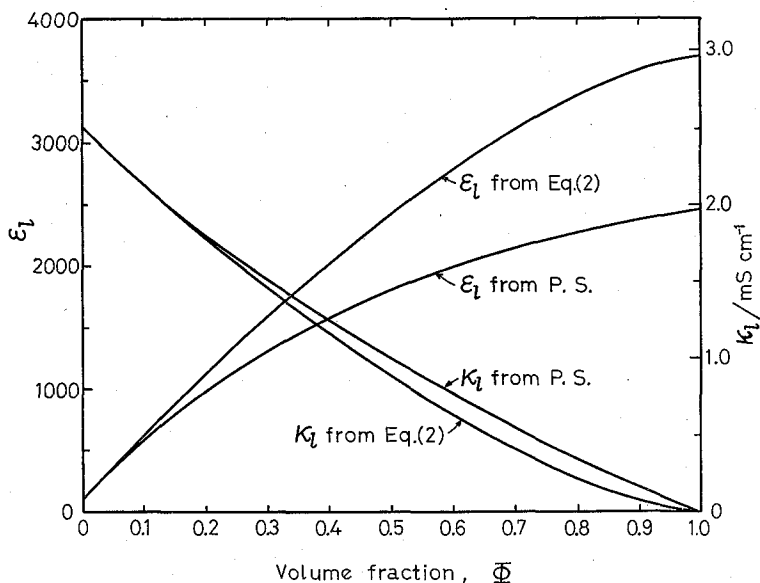


Fig. 4. Dependence of limiting relative permittivity ϵ_l and conductivity κ_l at low frequencies on volume fraction for the suspensions of shell-spheres calculated from Eq. (2) and from Pauly and Schwan's theory (P. S.). Phase parameters used for the calculations are the same as used in Fig. 2.

As readily known from the curve-fitting procedure, the values of Φ and ϵ_s are closely related to κ_l and ϵ_l respectively, the correlation being characteristic of respective theories. In Fig. 4 are compared the values of κ_l and ϵ_l calculated from Eq. (2) of the present theory and from Pauly and Schwan's theory with the same values of phase parameters. Marked differences of κ_l and ϵ_l values are found between the two theories. The phase parameters obtained by means of the curve-fitting procedure, therefore, are expected to vary from theory to theory used. The curve-fitting based on Pauly and Schwan's theory¹⁸⁾ was applied to the observed data of the erythrocyte suspension shown in Fig. 3, the phase parameters obtained being compared in Table III. Remarkable differences are found between the two theories with respect to the values of Φ , C_M , and ϵ_s as seen in the Table. A number of dielectric

Table III. Comparisons of Phase Parameters Obtained by Means of the Curve-Fitting Procedure

	Phase parameters estimated ^{a)}				
	Φ	C_M $\mu\text{F cm}^{-2}$	ϵ_s	ϵ_i	κ_l mS cm^{-1}
Eq. (2) of the present theory	0.540	0.777	4.39	69.0	2.84
Pauly and Schwan's theory ^{b)}	0.595	1.008	5.69	69.4	2.77

a) For the estimation from the curve-fitting procedure, the following values were used for both the theories: $\kappa_l=1.356 \text{ mS cm}^{-1}$, $\epsilon_l=2176$, $\epsilon_h=71.5$, $f_0=1.63 \text{ MHz}$, $D=4.75 \mu\text{m}$, $d=50 \text{ \AA}$, $\kappa_a=4.346 \text{ mS cm}^{-1}$, $\epsilon_a=77.1$.

b) The curve-fitting procedure is described in Reference 18.

data previously reported for biological suspensions await further reconsideration in the light of the present theory.

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